section. Apparently, evolution in section *Erythranthe* is proceeding principally by the accumulation of diverse genes in the various populations and species rather than by the accumulation of chromosomal differences.

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AN ANALYSIS OF VARIATION IN VIOLA NEPHROPHYLLA

Norman H. Russell and Frank S. Crosswhite[1]

The genus *Viola* is well known to be one of the more taxonomically "difficult" of the temperate angiosperms. Though in North America the "species" were sorted out in what appeared at the time to be a satisfactory manner (Brainerd, 1920), subsequent studies have shown that their limits are anything but clear. In particular regions it is possible to distinguish separate forms easily; in others there is so much morphological and ecological variability that distinct forms or even morphological types are very difficult to describe. Polyploidy, introgression, genetic drift in isolated populations, and other hypotheses have been used to explain this situation.

More important than the explanation of this morphological and physiological variation is the accurate and objective description of it. A method for the more objective comparison of units (individuals and aggregations of individuals) has been suggested by the senior author elsewhere (Russell, 1961, 1962) and is used in the present analysis and description. It consists of the preparation and correlation of multiple pair comparisons,

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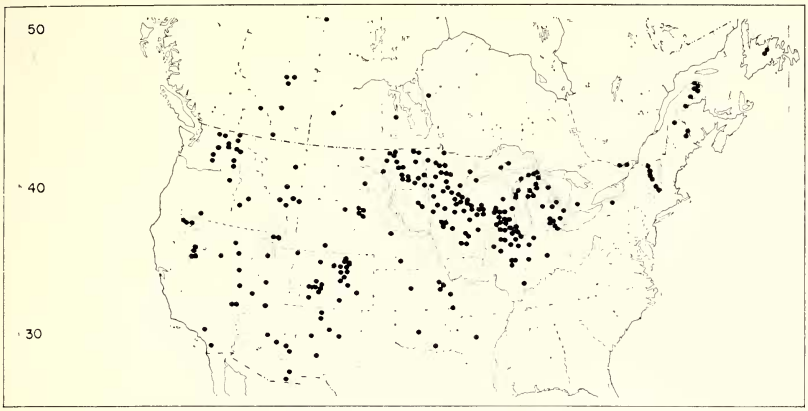

FIG. 1. Geographical distribution of *Viola nephrophylla* Greene.

using an index similar to that described by Anderson (1936), and is described in greater detail below.

Becker (1925), in the generally accepted classification of the genus *Viola,* recognized eleven sections, the most complex of which is the section *Plagiostigma.* This section was further divided into several subsections, the best known of which is the subsection *Boreali-americanae,* a group of perhaps twenty-six so-called "species," found exclusively in North America. Of these, only a single one, *Viola nephrophylla* Greene (Pittonia 3:144–45. 1896), occurs to any extent in the Rocky Mountains, the remainder being found almost exclusively in eastern and central North America. *Viola nephrophylla* has the largest range of any of the stemless blue violets. A map of its distribution (fig. 1) is based upon the examination of specimens from about sixty herbaria and represents the subjective decisions of the senior author on the basis of ten years of field and herbarium study of the genus *Viola.* Each dot, therefore, represents the place of collection of a herbarium specimen that, in his opinion, would key to *V. nephrophylla.* As is true of all such maps, it suffers from these subjective decisions and, therefore, must represent, perhaps, only an approximation to reality. The species, very likely, also occurs in Mexico, but we have not seen specimens from there. In many parts of its range, particularly where it does not grow with other kinds of stemless blue violets, it is morphologically distinct. However, in many situations in eastern North America, it is difficult to distinguish from related violets.

The habitat of *V. nephrophylla* varies widely in the Rocky Mountains. Usually we found it growing in moist, grassy, grazed fields, frequently in the shade of willows. Other populations were found along the shaded, sandy edges of canyon streams. In the eastern United States it grows both in open, grazed, poorly drained meadows and along the rocky shores of lakes in glaciated country. Figure 2 indicates the general appearance of this violet during the spring flowering period.

FIG. 2. *Viola nephrophylla,* spring appearance, × ⅔rds.

COLLECTION METHODS

Fifteen samples were taken by the junior author during the growing season of 1960, wherever they could be found, in rather extensive travels through Arizona and Colorado. Their locations are shown in figure 3. The number of specimens taken at each location varied from sixteen to fifty, depending upon the size of the local population. Ordinarily fifty specimens were obtained, but in some instances this was not possible. Plants were collected no closer together than six feet, to lessen the possibility of sampling two members of the same clone. They were usually measured or scored while fresh, but in some instances the plants were washed, pressed, and dried before examination. All the plants measured, and the measurement data, are deposited in the herbarium of Arizona State University.

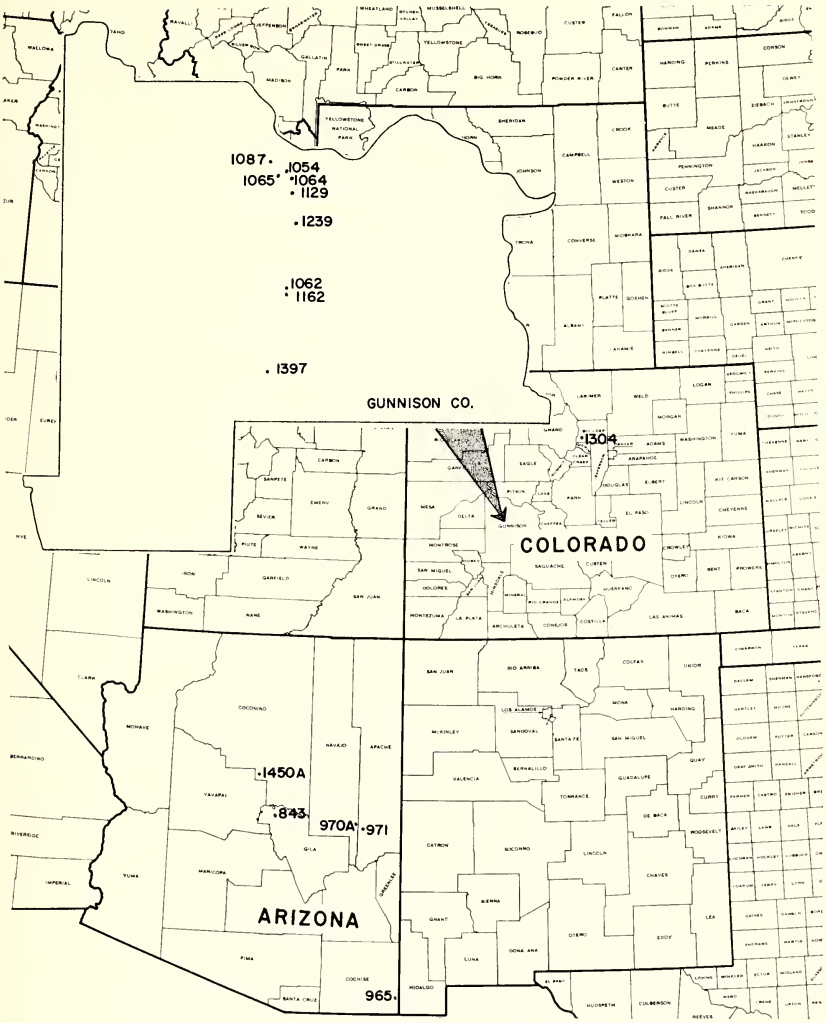

FIG. 3. Locations of population samples in Arizona and Colorado.

After a preliminary inspection of herbarium material, those characteristics showing the most conspicuous differences between individuals were chosen for analysis. The following characters were measured or scored as indicated:

1. Length of the lamina of the largest mature leaf.
2. Breadth of this lamina.
3. Distance from the apex of this lamina to one of the basal lobes.
4. The angle made by one-half of the apical margin of this leaf with the horizontal.

TABLE I. DISTRIBUTION (EXPRESSED AS PERCENTAGES) OF LAMINA LENGTH/BREADTH RATIOS, AND INDEX VALUES ASSIGNED FOR THE FIFTEEN POPULATIONS ANALYZED.

	Hybrid Index Scores								
	0		1		2				
Crosswhite Coll. No.	.50-.59	.60-.69	.70-.79	.80-.89	.90-.99	1.00-1.09	1.10-1.19	1.20-1.29	1.30-1.59
1450A	2.00	18.00	30.00	36.00	14.00	0	0	0	0
970A	0	0	0	68.75	31.25	0	0	0	0
965	12.50	56.25	31.25	0	0	0	0	0	0
971	0	0	35.00	40.00	17.50	7.50	0	0	0
1239	2.00	16.00	50.00	32.00	0	0	0	0	0
1162	0	26.67	40.00	23.33	10.00	0	0	0	0
843	0	0	16.00	48.00	24.00	8.00	0	0	4.00
1064	0	8.00	20.00	36.00	32.00	4.00	0	0	0
1065	0	0	12.00	60.00	18.00	8.00	2.00	0	0
1397	0	17.50	65.00	17.50	0	0	0	0	0
1304	0	4.00	36.00	44.00	16.00	0	0	0	0
1062	0	0	26.67	53.33	13.33	0	0	6.67	0
1129	0	4.00	14.00	30.00	48.00	4.00	0	0	0
1087	0	2.22	2.22	20.00	40.00	26.67	6.67	2.22	0
1054	0	0	4.00	24.00	28.00	24.00	16.00	4.00	0

5. Number of teeth on one-half the margin.
6. Pubescence of the upper lamina surface (scored as 0 = glabrous, 1 = slightly pubescent, 2 = moderately pubescent, and 3 = heavily pubescent).
7. Pubescence of the lower lamina surface (scored as indicated for the upper surface).
8. Pubescence of the margin of the lamina (scored as 0 = glabrous, 1 = hairy over half or more of its extent).
9. Pubescence of the petiole (scored as 0 = glabrous, 1 = 10 or more hairs present).

These leaf characteristics are those used by the senior author in studies on other stemless blue violets (Russell, 1955, 1956a, 1956b). It might have been desirable to measure other plant structures also, but either no conspicuous differences were noted in these or they were not present in all the samples. During the summer, when the majority of the samples were taken, no open flowers are produced, and, at times, no cleistogamous flowers or fruit. Rhizome differences, though present, were small and difficult to measure accurately.

Preparation of the Index

After compilation of the data (nine measurements or scores for each specimen), three ratios were calculated: lamina length/lamina breadth; lamina length/length from lamina apex to lobe; and lamina length/lami-

TABLE 2. INDEX VALUES ASSIGNED FOR EACH OF THE FIVE CHARACTERS USED IN THIS STUDY.

Character	Ranges in Characters		
	Value 0	Value 1	Value 2
Lamina length/ breadth ratio	.50-.69	.70-.89	.90-1.59
Lamina length/length to lobe ratio	.50-.69	.70-.79	.80-1.19
Lamina length/twice no. lamina teeth	.30-.59		.60-.99
Apical angle of lamina	20°-39°	40°-49°	50°-69°
Total lamina* pubescence	6-8	3-5	0-2

*Pubescence was scored on an orbitrary scale for both leaf surfaces, the margin, and the petiole. The value of 8 represented the greatest hairiness found, and that of 0 complete glabrousness.

na teeth. The distributions for each of the characteristics and ratios were then plotted for each sample. An example of one such distribution is shown in Table 1, for the lamina length/breadth ratio (an approximate measurement of overall shape). In the event real intra- or intersample differences were noted, index values (0, 1, and 2) were assigned to the extremes and median conditions found. This is illustrated in Table 1, and in Table 2 the index value assignments for all the characteristics used in the subsequent analyses are given.

In Table 3 the collections are arranged in order of the value of the mean index, in an attempt to reveal the existence of altitudinal or latitudinal clines. Total sample variation did not fall into any such pattern, although the Arizona samples generally had low values. Only a morphological "cline" can be shown, and we were unable to discover any geographical or ecological factor to which this could be definitely related.

The samples at opposite ends of Table 3 show considerable difference, enough so that if only these two extremes were known they might be called different "species" under present nomenclatural practices in plant taxonomy. As an example, the index distributions of two extreme collections have been plotted in figure 4, on a percentage basis. The differences

TABLE 3. SUMMARY OF DIFFERENCE INDEX SCORES FOR FIFTEEN POPULATION SAMPLES.

Crosswhite Coll. No.	Elev.	Latitude	No. Spec.	0	1	2	3	4	5	6	7	8	9	10	Mean	Location
1450A	5800'	35°	50		1	16	19	7	5	1	1				3.12	Oak Creek, Ariz.
970A	6000'	34°5'	16			4	5	2	4		1				3.63	Lakeside, Ariz.
965	5800'	31°50'	16			2	7	3	1	2	1				3.81	Portal, Ariz.
971	6000'	34°5'	40			1	7	16	6	9		1			4.48	Bog Creek, Ariz.
1239	9400'	38°50'	50			2	5	7	29	3	4				4.76	Cement Creek, Colo.
1162	8400'	38°30'	30			2	2	9	10	3	3		1		4.80	Almont, Colo.
843	6000'	34°20'	25			2	1	1	7	12	1	1			5.32	East Verde, Ariz.
1064	9200'	39°	25				2	5	6	6	4	2			5.44	Gothic, Colo.
1065	9300'	39°	50					10	10	12	13	3	2		5.90	Gothic, Colo.
1397	7900'	38°20'	40					6	7	12	7	8			6.10	Gunnison, Colo.
1304	9200'	40°	25					2	8	6	4	4	1		6.12	Nederland, Colo.
1062	8400'	38°30'	15					2	4	4	2	1	2		6.13	Almont, Colo.
1129	9000'	38°50'	50				1	5	7	11	11	9	6		6.54	Crested Butte, Colo.
1087	9500'	39°	41						2	5	22	4	7	1	7.29	Gothic, Colo.
1054	9400'	39°	25								3	5	10	7	8.84	Gothic, Colo.

between these two curves were analyzed in the following manner (Russell, 1961, 1962):

1. Percentage of the distance from the mode of one aggregation to the extreme value of the scale assigned to the other aggregation.
2. Percentage of the distance on the total index scale that the range of values for the aggregation does *not* cover.
3. Percentage of the total index for the range discontinuity (plus values) between the pair of samples or the total overlap (minus values) between them.
4. Percentage of the total index for the distance between the modes of the two curves.

These four descriptive features of the curves are analyzed in such a way as to show the greatest morphological separation of the two populations.

TABLE 4. MATRIX OF TOTAL DIFFERENCE INDICES FOR ALL SAMPLE COMPARISONS, TO BE READ HORIZONTALLY.

Crosswhite Coll. No.	*1450A*	*970A*	*965*	*971*	*1239*	*1162*	*843*	*1064*	*1065*	*1397*	*1304*	*1062*	*1129*	*1087*	*1054*	Mean
1450A	X	-29	-29	14	0	44	39	44	89	63	67	73	72	110	150	48.50
970A	-43	X	-33	13	0	50	42	50	100	71	75	81	81	122	167	55.43
965	-43	-83	X	13	0	50	42	50	100	71	75	81	81	122	167	51.86
971	-11	-43	-43	X	-15	1	-14	-8	51	15	26	32	32	78	123	16.00
1239	14	-17	-17	-29	X	0	-15	0	50	14	25	31	31	78	122	20.50
1162	10	-12	-12	-37	-37	X	-25	-19	0	0	-25	-19	-19	33	78	-6.00
843	39	14	14	-14	-15	-25	X	-50	-1	-42	1	-6	18	33	77	3.07
1064	49	29	29	13	0	-12	-15	X	14	-33	0	-7	-8	50	100	14.93
1065	100	88	88	63	63	38	38	21	X	-33	-34	-25	-50	-14	43	27.57
1397	87	72	72	44	43	25	15	-8	-33	X	1	-8	-7	43	100	31.86
1304	55	37	38	13	13	-12	13	-21	-50	-49	X	-25	-8	43	99	10.43
1062	67	50	50	25	25	0	12	-21	-67	-50	-67	X	-12	28	85	8.93
1129	66	50	50	25	25	0	0	-22	-29	-14	-15	-12	X	0	68	13.71
1087	110	100	100	78	78	55	56	44	-14	14	15	7	18	X	8	47.79
1054	190	190	190	168	167	145	145	144	100	128	128	121	118	66	X	142.86

Two separate analyses must be made, one for each population as compared to the other. The results of the analyses of the curves shown in figure 4 are:

Crosswhite 965 ..... 64/27/–9/55 *Crosswhite 1054*.....Total Index—137
Crosswhite 1054 ..... 82/64/–9/55 *Crosswhite 965*.....Total Index—192

Average Difference Index—164.5

Pictorialized scatter diagrams (figs. 5 and 6) illustrate the population differences further.

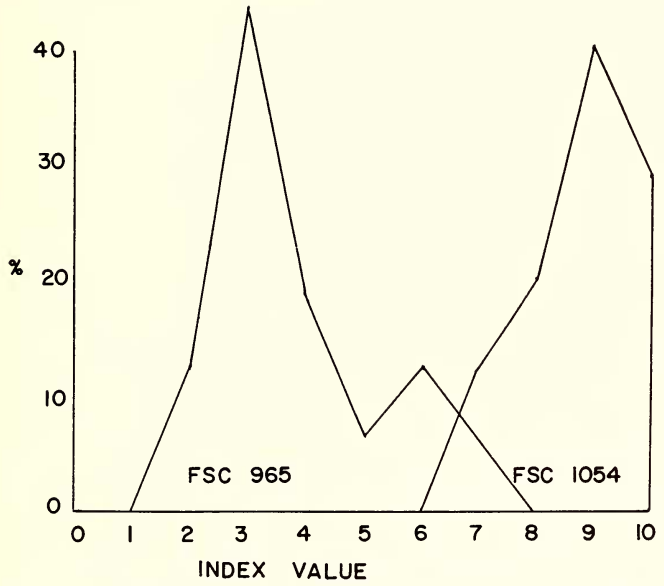

Fig. 4. Index distributions for two extreme populations; further explanation in text.

Total difference indices were computed for the other possible comparisons and were smaller than the above, grading down to minus values. They are shown in Table 4. When all the index distributions were converted to percentages and summed, a curve was obtained (fig. 7) which does not seem to indicate the presence of two or more types or "species" in this complex, but instead indicates a series of variable populations centering about an average overall morphological condition. A more penetrating analysis, using techniques such as those suggested by Rogers and Tanimoto (1960) might indicate whether or not more than one morphological type is present, and such an analysis is being planned. Other taxonomists have given specific rank to certain aberrant or differing types in the range of *Viola nephrophylla*; namely *V. arizonica* Greene, *V. cognata* Greene, *V. prionosepala* Greene, *V. McCabeiana* Baker, and *V. Clauseniana* Baker. We do not interpret the present descriptive data to support the recognition of different morphological types or "species."

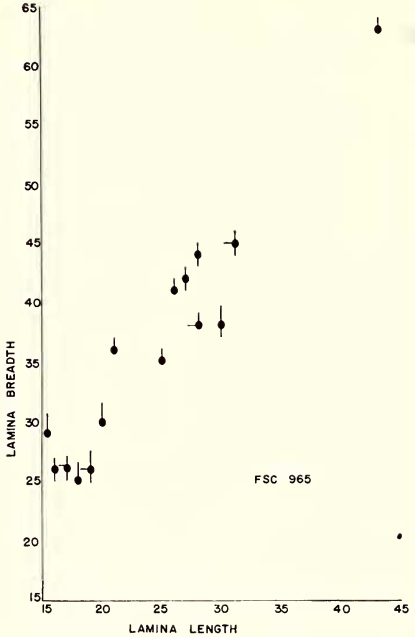

Fig. 5

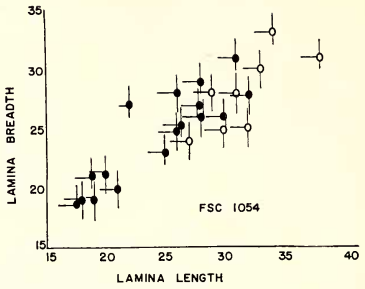

Fig. 6

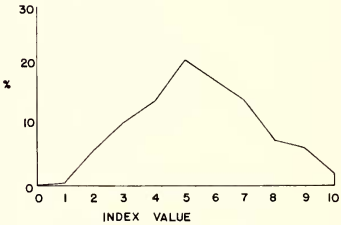

Fig. 7

Figs. 5–6. Pictorialized scatter diagrams of *Viola nephrophylla* populations: 5, *Crosswhite 965*; 6, *Crosswhite 1054*. Fig. 7. Distribution (converted to percentage values) of indices for all populations.

A sample collected in Oak Creek Canyon, Arizona, during September, 1960, deserves special mention (*Crosswhite 1450A*). The specimens differ (fig. 8) only in the relationship of lamina breadth to lamina length, the ratios exposing approximately equal numbers of plants with leaves wider than long and with leaves longer than wide. We have considered that the two differing regression lines may be explained either as the result of measurement errors, "juvenile" and mature leaves having been measured in equal numbers, or as the result of a nearly equal distribution of the members of a pair of alleles differently affecting lamina growth in the area collected. We have no data to test the second hypothesis, but have re-examined and remeasured the leaves and have, we believe, eliminated the possibility of error in choice of leaves.

Th taxonomic disposition of such aggregations of plants as that which we now call *Viola nephrophylla* still, of course, remains subject to the caprices of taxonomists who, under the present international rules, may justify their nomenclatural decisions by reference to their intuitive judgments. In this exploratory study, considering only a few samples in a part of the range of *V. nephrophylla*, we have submitted what we feel is good evidence for the rejection of the synonyms listed earlier for the Rocky

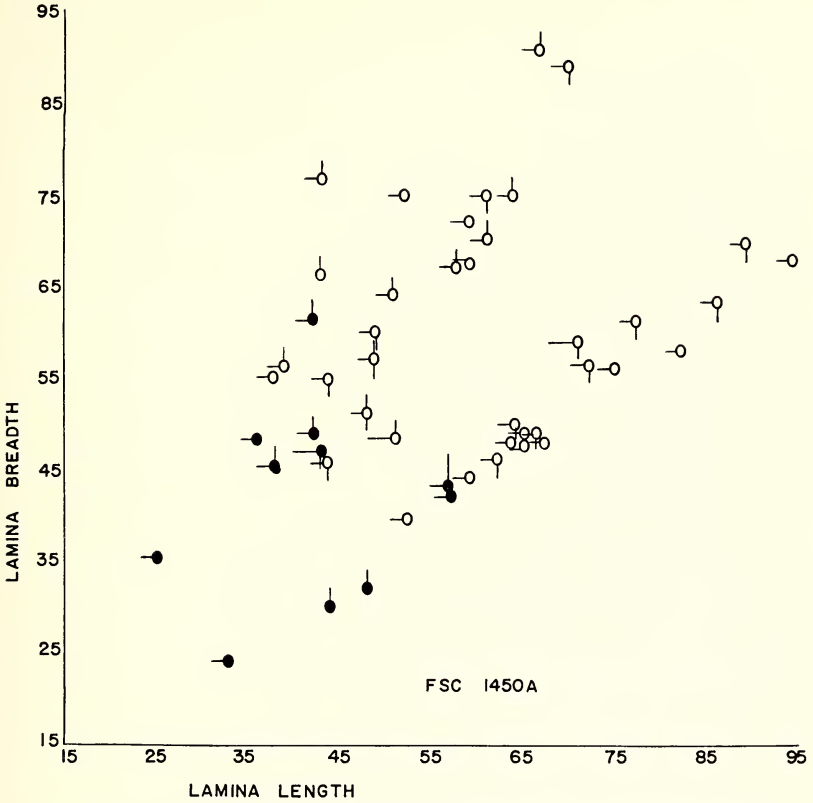

FIG. 8. Pictorialized scatter diagram for population sample, *Crosswhite 1450A*.

Mountain area. More elaborate studies are in progress on populations from the eastern and north-central part of the United States.

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